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SHORT COMMUNICATION

Study on the intermolecular complexation behavior between *p*-sulfonatocalix[4]arene with L-tyrosine

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Abstract The formation of the complexation behavior of host molecules water-soluble *p*-sulfonatocalix[4]arene (p-SCX4) with L-tyrosine (L-Tyr) guest molecule has been studied by spectrophotometric including fluorescence and nuclear magnetic resonance (NMR) spectroscopy. Experimental conditions including concentration of p-SCX4 and medium acidity were investigated in detail. The results showed that p-SCX4 forms 1:1 complexes with L-Tyr in water. Their stability constant determined by spectrofluorometric titration is 15761 L mol. Moreover, to obtain information about the binding mechanism of the interaction, ^{1}H NMR studies were carried out showing that the water-soluble p-SCX4 was found to be able to complex the aromatic L-Tyr, and the part of benzene ring of amino acid penetrated into the hydrophobic cavity of calix[4]arene and charged aliphatic chain of L-Tyr stick out of the cavity. In addition, the thermodynamic parameters for the complexation of p-SCX4 with L-Tyr were determined through Van't Hoff analysis. The obtained data further confirmed the binding mode of p-SCX4 with L-Tyr. The related mechanism is proposed to explain the complexation processes.

Keywords Spectrofluorometry · Aromatic L-Tyr · Water-soluble calix[4]arene · Host–guest interaction

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Introduction

The formation of host–guest complexes—the basis of supramolecular chemistry—especially in aqueous media is interesting not only from a mechanistic point of view but also from its possible applications, for example, in catalysis [1] and separation science [2]. The host compounds such as cyclodextrins [3] or calixarenes [4] are able to include small molecules, which result in remarkable variations in photophysical and photochemical properties of the guest because of the microenvironmental difference between the hosts interior and the solvent medium. Calixarene are bowl-shaped macrocycles host endowed with a cavity able to host a great variety of guests [5, 6], from apolar compounds such as fullerenes [7] to charged molecules such as metallic cations [8, 9], organic ammonium cations [10, 11], organic and inorganic anions [12, 13]. In addition, calixarene are described as 'macrocycles with almost unlimited possibilities' for their facile modification [14, 15]. Among these various calixarene derivatives, the chemistry of *p*-sulfonatocalixarenes are much more fascinating for less toxic than cyclodextrins and their water solubility [16], because many significant biological processes occur in aqueous solution such as enzyme catalysis [17], transport through membranes [18] and antibiotic activity [19, 20]. *p*-sulfonatocalixarenes possess the three-dimensional, flexible, π-electron rich cavities, and also can provide the additional anchoring points of sulfonate groups, which endows them versatile inclusion/complexation properties for charged and uncharged guest species both in the solid state and in water [21]. Furthermore *p*-sulfonatocalixarenes are demonstrated to promise biological, pharmaceutical and analytical applications owing to their perfect pre-organized structures and special binding characteristics [22].

The interaction of *p*-sulfonatocalixarenes with different biological activity molecules such as bio-macromolecules protein, is a topic of current interest in supramolecular and bioorganic chemistry [23]. The interactions of *p*-sulfonatocalixarenes with amino acids should provide important information on the mechanism of the binding of *p*-sulfonatocalixarenes to complex bio-macromolecules. Although there are a few reports that have focused on the interaction between them, the used study method are mostly limited NMR [24–26], microcalorimetric titration [27] RP-HPLC [28] and X-ray structure [29]. Arena et al. [24] reported that the interaction between amino acids with the water soluble p-SCX4 by NMR and the studies has shown that p-SCX4 forms 1:1 complexes with these amino acids in both near neutral (pH 7.3) and acidic solutions [25]. Doutéau-Guevel et al. [26] investigated the binding of dipeptides and tripeptides containing lysine or arginine with *p*-sulfonatocalixarenes in water by NMR and microcalorimetric studies [27]. The association constants, enthalpies and entropies of complexation have been determined. The structure of the complexes in solution has been studied by ^{1}H NMR spectroscopy. Kalchenko et al. comparatively studied the inclusion complexes of p-SCX4 with amino acids by reversed-phase high-performance liquid chromatography (RP-HPLC) [28] and ^{1}H NMR [28]. It was established that the formation of the inclusion complexes results in changes in the retention times of the amino acids and stability constants of the complexes were determined. These complexation of *p*-sulfonatocalixarene with the amino acids is generally governed by the hydrophobic, aromatic-aromatic and electrostatic interaction.

L-Tyr is one of three essential amino acids that carry an aromatic group. In this work, we have investigated host–guest interactions of p-SCX4 with L-Tyr (Fig. 1) in aqueous solutions by means of spectrofluorometric titration. The effect of pH and concentration of p-SCX4 was studied. ^{1}H NMR and Van't Hoff analysis were carried out to determine the possible mechanism for the binding reaction.

Experimental section

Apparatus

The fluorescence measurements were performed with a Hitachi Model F-4500 spectrofluorometer (Kyoto, Japan) equipped with a 150 Watt xenon lamp source, a thermostat bath and a quartz cells (1 × 1 cm). All pH values were measured with a pH_S-2 acidometre (The 2nd Instrument Factory of Shanghai, China). ^{1}H NMR spectra were recorded in D_2O on a Bruker—DRX—300 MHz spectrometer (Fällenden, Switzerland).

Reagents

The stock solution of 5.0×10^{-4} mol L^{-1} L-Tyr (Biological identification institute of Shanghai) and 1.0×10^{-2} mol L^{-1} p-SCX4 (Junsei chemical Co., Ltd.) were prepared by directly dissolving its powder in water. Phosphate buffer solutions were used to control the pH of the working media. Doubly distilled water was used throughout for all solutions.

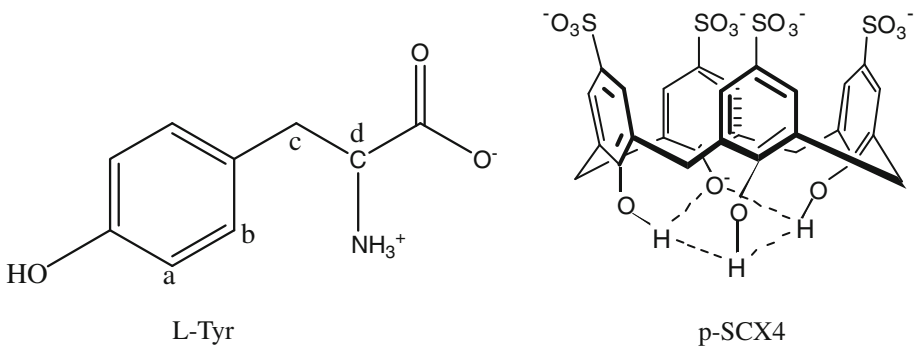
Procedure

Fluorescence spectroscopy

The 1.0 mL stock solution of 5.0×10^{-4} mol L^{-1} L-Tyr was transferred into a 10.0 mL volumetric flask and an appropriate amount of 0.010 mol L^{-1} p-SCX4 was added. The pH was controlled by a 0.5 mol L^{-1} phosphate buffer. The mixed solution was diluted to the final volume with the doubly distilled water and shaken thoroughly, then equilibrated for 30 min at room temperature.

^{1}H NMR spectroscopy

In the ^{1}H NMR experiments, which were conducted, changes in chemical shift, $\Delta\delta$, of signals in the ^{1}H NMR



L-Tyr p-SCX4

Fig. 1 Molecular structures of L-Tyr and p-SCX4

spectra of the L-Tyr. P-SCX4 was added directly into the solutions of L-Tyr in the NMR tubes and the resulting solutions was sonicated before recoding the spectra. All the spectra were recorded in 99.96% D_2O at 298 ± 0.1 K.

Results and discussion

Formation of complexes of L-Tyr and p-SCX4

Figure 2 illustrated the fluorescence spectral titration of p-SCX4 with 5.0×10^{-4} mol L^{-1} tyrosine guest in phosphate buffer solution of pH 7.0. The fluorescence maximum excitation and emission wavelengths of L-Tyr were at 281 and 307 nm, respectively. When the concentrations of p-SCX4 increased, the fluorescence intensity of L-Tyr gradually decreased. The maximum emission wavelength produced a small red shift from 307 to 312 nm and the corresponding excitation wavelength was slightly red shifted from 281 to 285 nm. The marked fluorescence quenching and the red shift proved the possible complex formation.

The binding constant (K) is a measure for the complexing capacity of a host compound (H) with a guest molecule (G). The binding constant can be obtained from the fluorescence data using a nonlinear curve-fitting approach [30] as shown below:

$$\Delta F = \frac{1}{2}\left\{\alpha\left([H]_0 + [G]_0 + \frac{1}{K}\right) - \sqrt{\alpha^2\left([H]_0 + [G]_0 + \frac{1}{K}\right)^2 - 4\alpha^2[H]_0[G]_0}\right\} \quad (1)$$

The nonlinear curve fitting function described the relation between the fluorescence intensity of L-Tyr at maximum emission wavelength and the different total concentration of the p-SCX4 in the case of 1:1 complexation. Where ΔF represents the change of the fluorescence intensity of L-Tyr with the addition of p-SCX4. $[H]_0$ and $[G]_0$ denote the initial concentrations of host p-SCX4 and guest L-Tyr, respectively. α is sensitive factor of the structure change of complexation composed of p-SCX4and L-Tyr at the interactive course. K is the binding constant. The correlation coefficients of the curves were 0.9999, indicating that the 1:1 complex stoichiometry between p-SCX4 and L-Tyr was formed.

Job plot is used to determine the stoichiometry of a binding event. It is also known as the Method of Continuous Variation which implied that the total molar concentration of the two binding partners is held constant, but their mole fractions are varied. The observable signal that is proportional to complex formation (such as fluorescence signal or absorption signal) is plotted against the mole fractions of these two components. The maximum on the plot corresponds to the stoichiometry of the two binding partners if sufficiently high concentrations are used. Figure 3 showed a representative Job's plot of p-SCX4 and L-Tyr. The maximum at 0.5 molar concentration also indicated the formation of a 1:1 ratio host–guest complex formed in aqueous solution [31].

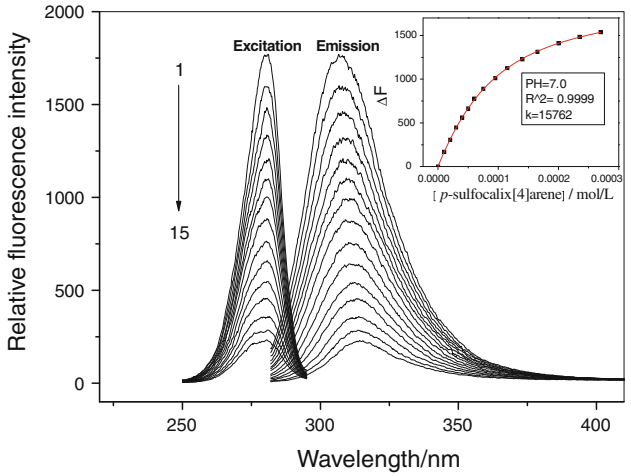


Fig. 2 Fluorescence spectral changes of L-Tyr (5×10^{-5} M) upon addition of various concentrations of p-SCX4 in phosphate buffer solution (pH 7.0) and the nonlinear least-square analysis (*inset*) of the differential intensity (ΔF) to calculate the complex stability constant (K). The concentration of p-SCX4 ($\times 10^{-5}$mol/L): 1, 0; 2, 1.0; 3, 2.0; 4, 3.0; 5, 4.0; 6, 5.0; 7, 6.0; 8, 7.5; 9, 9.5; 10, 11.5; 11, 14; 12, 16.5; 13, 20; 14, 23.5; 15, 27

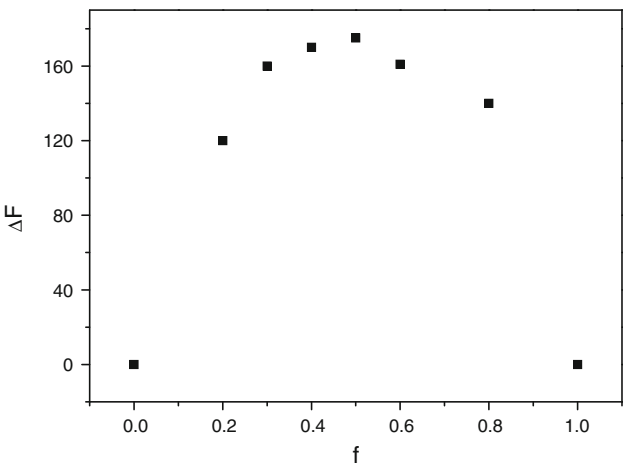


Fig. 3 Job's plot for the complexation of L-Tyr with p-SCX4 in phosphate buffer solution (pH 7. 0) The sum of the total concentration of interacting components is constant ([L-Tyr] + [p-SCX4]) = 5.0×10^{-5} M) and f is the mole fraction of p-SCX4 added

Influence of pH

The fluorescence spectra of L-Tyr have been studied in the pH range 2.0–10.0. There are three species (monocation, dipolar ion and monoanion) of L-Tyr. At different pH, the excitation and emission wavelengths of p-SCX4-L-Tyr red shifted contrast to the L-Tyr itself, which implying that the formation of host–guest complexation. In addition, the binding constant values were very sensitive to change of pH values (seen in Table 1). The complexation interaction of p-SCX4 with L-Tyr is the order: $K_{dipolar\ ion} > K_{nomoanion} > K_{monocation}$. The complexation of L-Tyr with p-SCX4 was pH-dependent since the ionization of L-Tyr in aqueous solution varied with pH and can be described as Scheme 1.

The sulfonic acid sites of p-SCX4 are completely deprotonated at pH 0.4 while the hydroxyl groups were completely undissociated [32, 33]. And the pKa value of the lower rim hydroxyl substituents of sulfonatocalix[4]arene were 3.34 and 11.5 [34]. In order to ensure that only the sulfonic acid moieties lose proton, the interaction between p-SCX4 and L-Tyr was studied at pH 2.0. At the pH 2.0, the monocation species with one positive charge of L-Tyr is predominant, and the electrostatic interaction of negatively charged p-SCX4 with positively monocation species of L-Tyr is possible. While as seen in Table 1, the binding constant did not decrease with increasing the negative charge of p-SCX4, which suggested that the electrostatic interaction may not be the only stabilizing factor for host–guest complex.

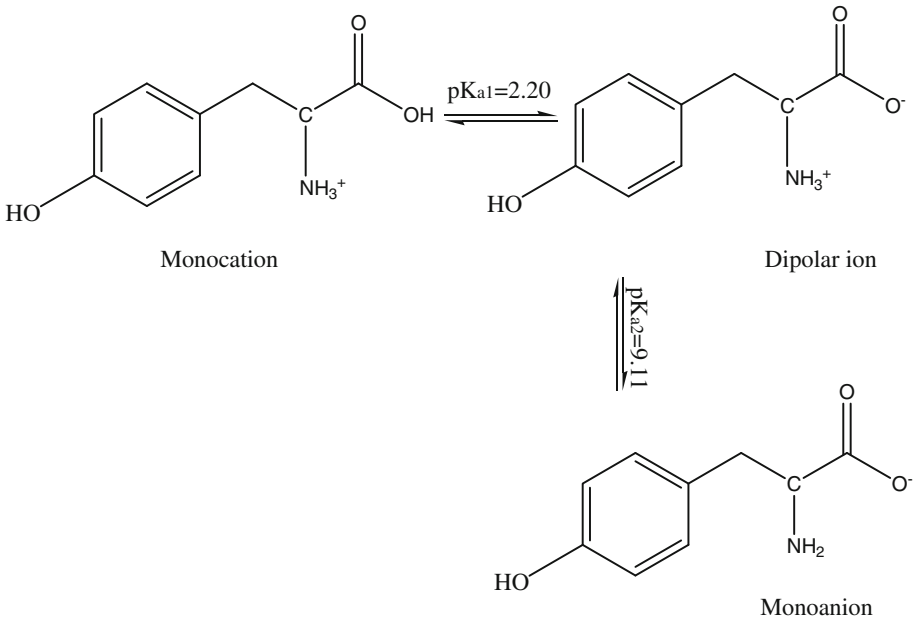
Table 1 Complex stability constants (K, L Mol^{-1}) for 1:1 intermolecular complexation of L-Tyr with p-SCX4 in different pH values

pH	2.0	4.0	5.5	7.0	8.5	10.0
n	1:1	1:1	1:1	1:1	1:1	1:1
K (L Mol^{-1})	3933	11318	14411	15761	15410	4750
R^2	0.9971	0.9993	0.9997	0.9999	0.9995	0.9999

Further inspection of the titration data revealed that the binding constant got to maximum in neutral aqueous. One of the phenolic OH groups of p-SCX4 is deprotonated in neutral or weak acid or weak basic aqueous solutions because the pKa value of the lower rim hydroxyl of p-SCX4 are 3.34 and 11.5, respectively. Whereas at pH 2.0 all the acidic phenol moieties do not lose proton. Coleman [34] had also reported that p-SCX4 exists as a penta-anion in neutral, which probably reflected that the deprotonation strengthened the hydrogen bonds among the phenolic hydroxyl groups allowing conformation flexibility for the calixarene ring (as shown in Fig. 1).

With increasing the pH until pH 11.5 or more, two of the phenolic OH groups of p-SCX4 are completely deprotonated. So, repulsive interaction of the two phenolic oxoanionic group O^- of the lower rim of p-SCX4 leads to the destructive hydrogen bonds and the little size of the cavity. Thus, in the pH range 4.0–8.5, the binding constant of p-SCX4 with L-Tyr is bigger than the pH 2.0 and 10.0. The different binding constant of p-SCX4 with L-Tyr at the different pH is mainly attributed to the host size and conformation change, shown in Table 1, originates from the strength of the CH-π/π–π electronic interaction between the host and guest aromatic rings.

Scheme 1 The conversion of three species of L-Tyr in different pH



The thermodynamic parameters of inclusion complexation

The determination of thermodynamic parameters, here were three parameters in the complexation process, the Gibbs free energy change (ΔG), the enthalpy changes (ΔH) and the entropy changes (ΔS). The thermodynamic parameters for the complexation of the guest with host compound are influenced by several factors: good size-fit, CH-π/π–π, hydrophobic, spatial conformation, hydrogen bonding, and electrostatic attraction as well as van der Waals, and so on. The binding constants (K) of the complexation of p-SCX4 with L-Tyr were determined via spectrofluorometric titration at various temperatures ranging from 293.0 to 323.0 K at pH 7.0. The complexation thermodynamic parameters were obtained by the slope and ordinate-intercept of Van't Hoff equation [30]:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

The ΔG was calculated from the following relationship:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

Figure 4, by fitting the data of Table 2, shows that assumption of near constant ΔH was justified. The negative free energy (ΔG) suggested that the inclusion process proceeded spontaneously. Moreover, all the values of ΔH and ΔS of the resulting complexes are positive. These results indicate that the complexation of p-SCX4 with L-Tyr examined is driven predominantly by the favorable entropy changes, typically showing large positive entropy changes ($T\Delta S$ = 47–52 kJ mol^{-1}) and somewhat smaller positive enthalpy changes (ΔH = 24.54 kJ mol^{-1}). The entropy changes originated from the entropic gain from the rearrangement of water molecules originally surrounding the host and guest molecules, and the entropic loss from the

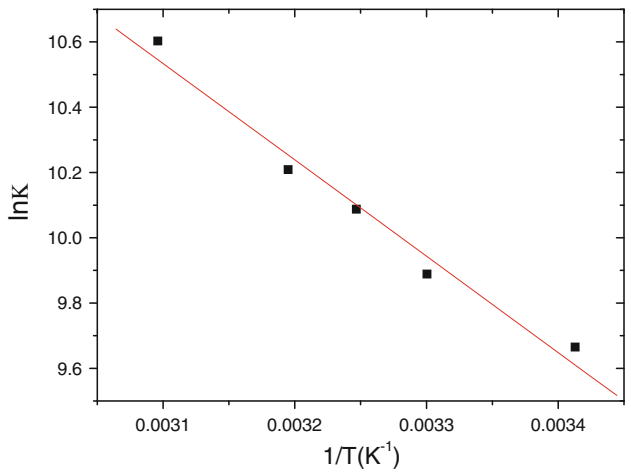


Fig. 4 Van't Hoff plot, pH 7.0, c (L-Tyr) = 5 × 10^{-5} mol/L

Table 2 Complex stability constants (K, L/Mol) and thermodynamic parameters for 1:1 intermolecular complexation of L-Tyr with p-SCX4 in phosphate buffer solution (pH = 7.0)

	T (K)	K (L/mol)	ΔH (kJ/mol)	ΔG (kJ/mol)	$T\Delta S$ (kJ/mol K)
pH = 7.0	293.0	15761	24.54	−23.41	47.95
	303.0	19709		−25.05	49.59
	308.0	24034		−25.86	50.40
	313.0	27137		−26.68	51.22
	323.0	40256		−28.32	52.86

decrease in the motion freedom upon the complexation [35]. So, the possible explanation for the complexation of the large entropy-driven is that, both L-Tyr and p-SCX4 are heavily solvated. Thus, hydrophobic interaction is one influence factor for complexation process.

^{1}H NMR spectra

NMR spectroscopy is indeed the most powerful tools for the study of formation of inclusion complex between hosts and a variety of guest molecules, especially the interaction mechanism [26, 28]. Arena and coworkers [36] detailed studied of the complexation of p-SCX4 with five different amino acids not including L-Tyr using both NMR and calorimetry. ^{1}H NMR spectra of L-Tyr, p-SCX4 and its complex at room temperature were shown in Fig. 5. Some representative results are listed in Table 3. L-Tyr has four types of hydrogen: H_a, H_b, H_c and H_d. When p-SCX4 complexed with L-Tyr, it was obvious that H_d of L-Tyr moved significant downfield in the p-SCX4, which is the result of the deshielding effect by the p-SCX4. This phenomenon may attribute to the effect between the anionic sulfonyl group–SO_3^- of p-SCX4 and the cationic amino group–$NH_3^+$of L-Tyr. While H_a H_b and H_c proton displayed little upfield frequency changes to some extent in the p-SCX4, which illustrated the shielding effect of the protons by the cavity of p-SCX4. These different magnitudes of the chemical shift changes suggested that: exposure the H_d of L-Tyr out the cavity of p-SCX4; H_a H_b and H_c inside the cavity. That is to say the part of benzene ring of amino acid penetrated into the hydrophobic cavity of p-SCX4and charged aliphatic chain of L-Tyr stick out of the cavity.

Conclusion

Spectrofluorometric titration and ^{1}H NMR investigation has demonstrated the complexation interaction between L-Tyr and p-SCX4. The formation constants, binding ratio, enthalpy and entropy of complexation were evaluated by

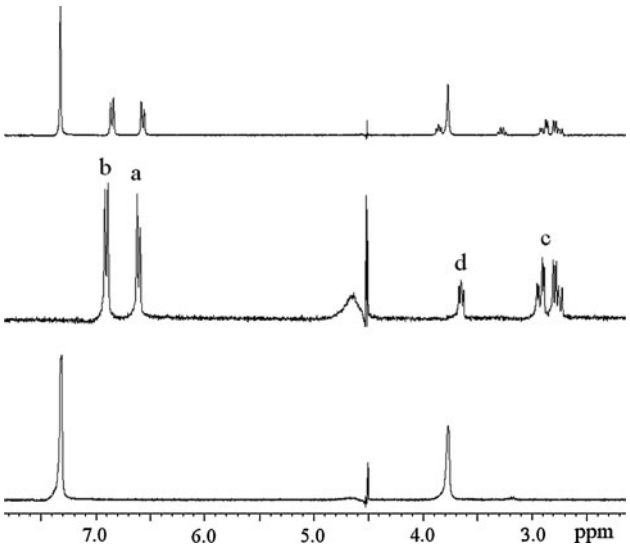


Fig. 5 ^{1}H NMR spectra of p-SCX4 complexed with L-Tyr, L-Tyr and p-SCX4 from top to bottom at 24 °C at pH 7.0 in D_2O at 300 MHz. (c (L-Tyr) = 5×10^{-5} mol L, c (p-SCX4) = 5×10^{-5} mol L, the ration of them is 1:1)

Table 3 ^{1}H NMR spectra of p-SCX4 complexed with L-Tyr, L-Tyr and p-SCX4 from top to bottom at 24° at pH 7.0 in D_2O at 300 MHz. (c (L-Tyr) = 5×10^{-5} mol/L, c (p-SCX4) = 5×10^{-5} mol/L)

Proton	δ_{free}/ppm	δ_{com}/ppm	$\triangle\delta^a$/ppm
H_a of L-Try	6.608	6.577	−0.031
H_b of L-Try	6.905	6.861	−0.044
H_c			
H_{c1} of L-Try	2.919	2.912	−0.007
H_{c2} of L-Try	2.764	2.739	−0.025
H_d of L-Try	3.654	3.859	0.205

[a] $^{\Delta}\delta = \delta_{com}$-$\delta_{free}$

spectrofluoreometric titration. The fluorescence intensity of the L-Tyr gradually decreased upon the addition of p-SCX4, accompanying with bathochromic shifts of the emission spectrum. From ^{1}H NMR, it was concluded that the benzene ring protons of L-Tyr showed a remarkable upfield shift, benzene apolar group was partially enclosed into the apolar hydrophobic cavity of p-SCX4 cavity and the charged group of the L-Tyr sticked out of the cavity. The possible mechanism involved in the complexation between L-Tyr and p-SCX4 may be a combination of hydrophobic interaction and electrostatic interaction. Moreover, p-SCX4 is conformationally very flexible and it could include L-Tyr to form host–guest complexes and alter the physical and chemical properties of L-Tyr. Therefore, there will be potential applications in the further biological pharmaceutical development.

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